

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Chambon *et al.*

Appl. No. To be assigned

Filed: Herewith

For: **Transcriptional Intermediary
Factor-2**

Confirmation No.:

Art Unit: To be assigned

Examiner: To be assigned

Atty. Docket: 1383.0130002

Preliminary Amendment

Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of prosecution, please amend the application as follows. This Amendment is provided in the following format:

(A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;

(B) Starting on a separate page, appropriate remarks and arguments.

37 C.F.R. § 1.111 and M.P.E.P. 714; and

(C) Starting on a separate page, a marked-up version entitled: “Version with markings to show changes made.”

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

09042256-042601

Amendments

In the Specification:

Please substitute the following paragraphs/sections for the pending paragraphs/sections.

Substitute the paragraph beginning on page 1, line 3, with the following paragraph:

This application is a divisional of U.S. Appl. No. 08/891,640, filed July 11, 1997; said 08/891,640 claims priority to U.S. Appl. No. 60/021,247, filed July 12, 1996, the disclosures of both of which are herein incorporated by reference.

Substitute the paragraph beginning on page 8, line 10, with the following paragraph:

Figure 1(a-n). The nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of the transcriptional intermediary factor-2 (TIF2) protein. This protein has a deduced molecular weight of about 160 kDa. The amino acid sequence of the functional coactivator TIF2.1 protein fragment is shown from amino acid residue 624 to residue 1287.

Substitute the paragraph beginning on page 9, line 27, with the following paragraph:

Figure 3(a-c). Amino acid sequence of TIF2: homology with SRC-1 indicates the existence of a novel family of NR mediators.

Substitute the paragraph beginning on page 10, line 1, with the following paragraph:

(a-b) Alignment and amino acid sequences of TIF-2 (SEQ ID NO:2) and the steroid receptor coactivator SRC-1 (SEQ ID NO:3) (Onate, S.A. *et al.*, *Science* 270:1354-1357

0942256-042601
106240-95224860

(1995)). Two charged clusters rich in acidic and basic amino acid residues, three serine/threonine (S/T)-rich regions and one glutamine-rich region are highlighted. The N-terminal charged cluster contains putative bipartite nuclear localization signals (NLSs) (overlined). The regions encoding TIF2.1 (amino acids 624 to 1287; functional coactivator fragment) and dnSRC-1 (amino acids 865 to 1061; dominant negative fragment) are indicated. An asterisk identifies the TIF2 stop codon. Note that TIF2.1 and dnSRC-1 do not overlap, indicating that dnSRC-1 may possibly contain a NR-interacting region distinct from that of TIF2.1.

Substitute the paragraph beginning on page 10, line 12, with the following paragraph:

(c) Schematic comparison of TIF2 and SRC-1. Percent identities (similarities in parentheses) of homologous regions are indicated. The N-terminal charged cluster harboring the putative NLS and the C-terminal S/T-rich region of TIF2 are not, or only weakly, conserved in SRC-1.

Substitute the paragraph beginning on page 13, line 13, with the following paragraph:

Figure 6 (a-c). Schematic representation of reporter genes (A) and receptor expression vectors (B-C) (see the Materials and Methods section of Nagpal *et al.*, *EMBO J* 12(6):2349-2360 (1993) for a detailed description of construction). Sequences of mCRBP II (SEQ ID NO:11) and mCRBP II(17m-ERE)/CAT (SEQ ID NO:11) are indicated. Minus and plus numbers are with respect to the RNA start site (+1). In (B-C), the various regions (A-F) of wild-type RARs and RXRs, as well as their truncation mutants, substitution mutants and chimeric receptor constructs are schematically represented (not to scale) (see Zelent *et al.*,

09842256-042601

Nature 339:714-717 (1989); Leid *et al.*, *Trends Biochem. Sci.* 17:427-433 (1992); Leid *et al.*, *Cell* 68:377-395 (1992); Nagpal *et al.*, *Cell* 70:1007-1019 (1992); and Allenby *et al.*, *Proc. Natl. Acad. Sci. USA* 90:30-34 (1993)). Numbers indicate the amino acid positions in the wild-type receptor. The positions of the amino acid substitutions are indicated with an arrow.

Substitute the paragraph beginning on page 15, line 8, with the following paragraph:

Figure 8(a-f). Mapping of the TIF2 nuclear receptor interacting domain (NID).

Substitute the paragraph beginning on page 15, line 25, with the following paragraph:

(e-f) Effect of TIF2 NID point mutations on stimulation of NR AF-2 activity. Cos-1 cells were cotransfected with 1 µg of the (17m)₅-TATA-CAT reporter, 0.2 µg of GAL-hERα(EF) or GAL-mRXRα(DE), and 2.5 µg of the TIF2.1 wildtype or mutated fragments, as indicated. The reporter gene activation relative to the TIF2.1 wildtype activity and in presence of 10⁻⁶ M estradiol (E2) or all-*trans-retinoic* acid (RA), respectively, is indicated for each mutant (black bars); for comparison, *in vitro* binding of the respective mutants relative to TIF2.1 wildtype binding in presence of ligand is indicated by the white bars. Each bar represents the mean value obtained from at least three (interaction) or at least four (transactivation) experiments, respectively; standard deviations are indicated. Note that the absolute values for TIF2.1 wildtype activity varied by ±16% when cotransfected with GAL-hERα(EF) and by ±34% when cotransfected with GAL-mRXRα(DE). In the *in-vitro*-interaction assays, the affinity of the TIF2.1 wildtype standard varied by less than ±25%. Expression levels of TIF2 mutants in the cells were verified by Western blot (not shown)

09842256, 042601

with mouse monoclonal antibody 3Ti3F1, which is directed against an epitope outside the mutated area.

Substitute the paragraph beginning on page 20, line 25, with the following paragraph:

In another aspect, the invention provides isolated nucleic acid molecules encoding the TIF2 polypeptide having an amino acid sequence as encoded by the cDNA clone deposited as ATCC Deposit No. 97612 on June 14, 1996 (American Type Culture Collection, (ATCC) 10801 University Boulevard, Manassas, Virginia 20110-2209). The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) or the nucleotide sequence of the TIF2 cDNA contained in the above-described clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated nucleic acid molecules, preferably DNA molecules, are useful as probes for gene mapping by *in situ* hybridization with chromosomes and for detecting expression of the TIF2 gene in human tissue, for instance, by Northern blot analysis.

In the Claims:

Please cancel claims 1-10 without prejudice or disclaimer.

094236 1042601

Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 11-20 are pending in the application. The specification was amended to update the priority information, to comport the description of the drawings in the specification with the formal drawings, and to add the address for the ATCC. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Prompt and favorable consideration of this Amendment is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Andrea Jo Kamage

Andrea Jo Kamage
Agent for Applicants
Registration No. 43,703

Date: April 26, 2001

1100 New York Avenue, N.W.
Suite 600
Washington, D.C. 20005-3934
(202) 371-2600

0904236.042601

Version with markings to show changes made

The paragraph beginning on page 1, line 3:

This application is a divisional of U.S. Appl. No. 08/891,640, filed July 11, 1997;
said 08/891,640 claims priority to U.S. Appl. No. 60/021,247, filed July 12, 1996, the
disclosures of both of which [is] are herein incorporated by reference.

The paragraph beginning on page 8, line 10:

Figure 1(a-[b]n). The nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of the transcriptional intermediary factor-2 (TIF2) protein. This protein has a deduced molecular weight of about 160 kDa. The amino acid sequence of the functional coactivator TIF2.1 protein fragment is shown from amino acid residue 624 to residue 1287.

The paragraph beginning on page 9, line 27:

Figure 3(a-[b]c). Amino acid sequence of TIF2: homology with SRC-1 indicates the existence of a novel family of NR mediators.

The paragraph beginning on page 10, line 1:

(a-b) Alignment and amino acid sequences of TIF-2 (SEQ ID NO:2) and the steroid receptor coactivator SRC-1 (SEQ ID NO:3) (Onate, S.A. *et al.*, *Science* 270:1354-1357 (1995)). Two charged clusters rich in acidic and basic amino acid residues, three serine/threonine (S/T)-rich regions and one glutamine-rich region are highlighted. The N-terminal charged cluster contains putative bipartite nuclear localization signals (NLSs)

0042256.042601
109240" 95224860

(overlined). The regions encoding TIF2.1 (amino acids 624 to 1287; functional coactivator fragment) and dnSRC-1 (amino acids 865 to 1061; dominant negative fragment) are indicated. An asterisk identifies the TIF2 stop codon. Note that TIF2.1 and dnSRC-1 do not overlap, indicating that dnSRC-1 may possibly contain a NR-interacting region distinct from that of TIF2.1.

The paragraph beginning on page 10, line 12:

[(b)] (c) Schematic comparison of TIF2 and SRC-1. Percent identities (similarities in parentheses) of homologous regions are indicated. The N-terminal charged cluster harboring the putative NLS and the C-terminal S/T-rich region of TIF2 are not, or only weakly, conserved in SRC-1.

The paragraph beginning on page 13, line 13:

Figure 6 (a-[b]c). Schematic representation of reporter genes (A) and receptor expression vectors (B-C) (see the Materials and Methods section of Nagpal *et al.*, *EMBO J* 12(6):2349-2360 (1993) for a detailed description of construction). Sequences of mCRBP II (SEQ ID NO:11) and mCRBP II(17m-ERE)/CAT (SEQ ID NO:11) are indicated. Minus and plus numbers are with respect to the RNA start site (+1). In (B-C), the various regions (A-F) of wild-type RARs and RXRs, as well as their truncation mutants, substitution mutants and chimeric receptor constructs are schematically represented (not to scale) (see Zelent *et al.*, *Nature* 339:714-717 (1989); Leid *et al.*, *Trends Biochem. Sci.* 17:427-433 (1992); Leid *et al.*, *Cell* 68:377-395 (1992); Nagpal *et al.*, *Cell* 70:1007-1019 (1992); and Allenby *et al.*, *Proc. Natl. Acad. Sci. USA* 90:30-34 (1993)). Numbers indicate the amino acid positions

0942256-04601
109240-9524860

in the wild-type receptor. The positions of the amino acid substitutions are indicated with an arrow.

The paragraph beginning on page 15, line 8:

Figure 8(a-[e]f). Mapping of the TIF2 nuclear receptor interacting domain (NID).

The paragraph beginning on page 15, line 25:

(e-f) Effect of TIF2 NID point mutations on stimulation of NR AF-2 activity. Cos-1 cells were cotransfected with 1 μ g of the (17m)₅-TATA-CAT reporter, 0.2 μ g of GAL-hER α (EF) or GAL-mRXR α (DE), and 2.5 μ g of the TIF2.1 wildtype or mutated fragments, as indicated. The reporter gene activation relative to the TIF2.1 wildtype activity and in presence of 10⁻⁶ M estradiol (E2) or all-*trans-retinoic* acid (RA), respectively, is indicated for each mutant (black bars); for comparison, *in vitro* binding of the respective mutants relative to TIF2.1 wildtype binding in presence of ligand is indicated by the white bars. Each bar represents the mean value obtained from at least three (interaction) or at least four (transactivation) experiments, respectively; standard deviations are indicated. Note that the absolute values for TIF2.1 wildtype activity varied by $\pm 16\%$ when cotransfected with GAL-hER α (EF) and by $\pm 34\%$ when cotransfected with GAL-mRXR α (DE). In the *in-vitro*-interaction assays, the affinity of the TIF2.1 wildtype standard varied by less than $\pm 25\%$. Expression levels of TIF2 mutants in the cells were verified by Western blot (not shown) with mouse monoclonal antibody 3Ti3F1, which is directed against an epitope outside the mutated area.

09042336 "042501

The paragraph beginning on page 20, line 25:

In another aspect, the invention provides isolated nucleic acid molecules encoding the TIF2 polypeptide having an amino acid sequence as encoded by the cDNA clone deposited as ATCC Deposit No. 97612 on June 14, 1996 (American Type Culture Collection, (ATCC) [Rockville, MD] 10801 University Boulevard, Manassas, Virginia 20110-2209). The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) or the nucleotide sequence of the TIF2 cDNA contained in the above-described clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated nucleic acid molecules, preferably DNA molecules, are useful as probes for gene mapping by *in situ* hybridization with chromosomes and for detecting expression of the TIF2 gene in human tissue, for instance, by Northern blot analysis.

Claims 1-10 were cancelled.

094225-042601
109240" 9524350